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SUSCEPTIBILITY

Fluconazole Resistance in Isolates of Uncommon Pathogenic Yeast Species from the United Kingdom

Andrew M. Borman,[a] Julian Muller,[b]* Jo Walsh-Quantick,[c] Adrien Szekely,[a] Zoe Patterson,[a] Michael D. Palmer,[a] Mark Fraser,[a] Elizabeth M. Johnson[a]

[a]PHE UK National Mycology Reference Laboratory, Science Quarter, Southmead Hospital, Bristol, United Kingdom
[b]Bristol Medical School, University of Bristol, Bristol, United Kingdom
[c]School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom

ABSTRACT The triazole drug fluconazole remains one of the most commonly prescribed antifungal drugs, both for prophylaxis in high-risk patients and also as a second-line treatment option for invasive *Candida* infections. Established susceptibility profiles and clinical interpretive breakpoints are available for fluconazole with *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, and *Candida parapsilosis*, which account for the majority of infections due to pathogenic yeast species. However, less common species for which only limited susceptibility data are available are increasingly reported in high-risk patients and from breakthrough infections. The UK National Mycology Reference Laboratory performs routine antifungal susceptibility testing of clinical isolates of pathogenic yeast submitted from across the United Kingdom. Between 2002 and 2016, ~32,000 isolates were referred, encompassing 94 different yeast species. Here, we present fluconazole antifungal susceptibility data generated using a CLSI methodology over this 15-year period for 82 species (2,004 isolates) of less common yeast and yeast-like fungi, and amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and anidulafungin, with members of the *Nakaseomyces* clade (*C. glabrata*, *Candida nivariensis*, and *Candida bracarensis*). At least 22 different teleomorph genera, comprising 45 species, exhibited high MICs when tested with fluconazole (>20% of isolates with MICs higher than the clinical breakpoint [≥8 mg/liter] proposed for *C. albicans*). Since several of these species have been reported anecdotally from breakthrough infections and therapeutic failures in patients receiving fluconazole, the current study underscores the importance of rapid and accurate yeast identification and may aid clinicians dealing with infections with rarer yeasts to decide whether fluconazole would be appropriate.

KEYWORDS *Candida*, MIC, antifungal resistance, fluconazole, rare yeast species

Invasive *Candida* infections continue to be associated with high rates of morbidity and mortality, particularly in immunocompromised hosts (1, 2), where early diagnosis and appropriate therapy are vital to improve outcomes (1, 3). While most studies agree that *Candida albicans* remains the principal agent of nosocomial yeast infections, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lusitaniae*, and *Candida krusei* have emerged over recent years as significant opportunistic pathogens (1, 2, 4). However, in total, in excess of 150 yeast spp. from *Candida* and various other genera have to date been reported from mammalian infections (5–11), in part due to the expanded use of antifungal agents with activity against the more common *Candida* species.

It is now well established that differences in antifungal susceptibility exist between the more common *Candida* species (8, 12) and species-specific interpretive clinical breakpoints (CBPs) and epidemiological cutoff values (ECVs) based on wild-type distri-

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Address correspondence to Andrew M. Borman, Andy.Borman@nbt.nhs.uk.

* Present address: Julian Muller, Barts Health NHS Trust, London, United Kingdom.

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butions have been proposed for a number of these *Candida* species-antifungal agent combinations by both the Clinical and Laboratory Standards Institute (CLSI; reviewed in references 13 and 14) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; reviewed in reference 15). Thus, for such species, rapid and robust identification of the infecting species can be used to predict likely antifungal resistance of the majority of the species. For those species that are relatively rarely encountered in the clinical setting, such data are lacking. According to CLSI criteria (EUCAST criteria are slightly different), the establishment of ECVs requires the analysis of at least 100 independent isolates of a particular species, with MIC values obtained from at least three independent centers (reviewed in reference 13), and the development of CBPs is hindered by the lack of sufficient clinical trial data for rare or emerging pathogens. In such instances, where therapeutic options can be inferred at best from limited anecdotal case reports, the analysis of MIC distributions from longitudinal studies can prove useful in identifying antifungal agent-organism combinations where MIC ranges are elevated compared to other species (7, 8, 15, 16).

Fluconazole (FLC), a triazole antifungal with fungistatic activity against many *Candida* species, is one of the most frequently prescribed antifungal drugs (17). It is widely employed prophylactically in high-risk patients and especially in neonates (3, 18), is the preferred treatment for *Candida* osteomyelitis and infections of the eyes and urinary tract (3), and is an appropriate step-down therapy for candidemia, disseminated hepatosplenic candidiasis, and central nervous system and intravascular infections in patients infected with isolates that are unlikely to be fluconazole resistant or are without prior fluconazole exposure (3). CBPs have been developed for fluconazole with several common *Candida* species (19), and ECVs have been proposed for some less common species (20). In addition, the available data support the use of fluconazole based on the results of *in vitro* susceptibility testing for several of the most common *Candida* species (21). Despite this growing evidence base, data concerning fluconazole susceptibility for rare species of pathogenic yeasts are limited. Here, we have attempted to partially redress this limitation, and we present the results of 15 years of fluconazole *in vitro* susceptibility testing on a panel of >32,000 yeast isolates, which includes over 80 rare or emerging species. In addition, MICs for amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and anidulafungin with members of the *Nakaseomyces* clade (*C. glabrata*, *Candida nivariensis*, and *Candida bracarensis*) are also presented.

RESULTS

During the period of 2002 to 2016, the MRL received 31,964 isolates of pathogenic yeast, referred after their isolation from clinical samples across the United Kingdom for antifungal susceptibility testing against at least fluconazole (Table 1). In agreement with many other reports concerning species prevalence in candidiasis (1, 2, 4–8), the most common organisms referred to the MRL in descending order of prevalence were *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *Pichia kudriavzevii* (*C. krusei*), *Saccharomyces cerevisiae*, *Clavispora* (*Candida*) *lusitaniae*, *Cryptococcus neoformans*, and *Candida dubliniensis*, which together accounted for 95.3% (30,476/31,964) of all isolates. The remaining isolates encompassed an additional 85 species distributed between the anamorph "genus" *Candida* and many additional teleomorph yeast genera in both the Ascomycota and Basidiomycota (Table 1).

MIC data were assembled for all yeast species that were less common than *C. dubliniensis* (Table 1) which had been referred to the laboratory for fluconazole antifungal susceptibility testing (Tables 2 and 3). In all tests, the MICs of the control reference strains were within the accepted limits (data not shown), and MIC distributions with quality control (QC) strains were remarkably consistent over the time period of the current study (see Table S1 in the supplemental material). Since CBPs and ECVs are not available for the less common organisms, we employed the *C. albicans* fluconazole CBP as a means of identifying isolates/species with elevated fluconazole MICs/MIC ranges (13, 14, 19, 20). Thus, potentially resistant isolates were identified as

TABLE 1 Yeast species submitted to the MRL between 2002 and 2016 for fluconazole susceptibility testing[a]

Organism (anamorph/previous name)	Prevalence of isolates received from 2002–2016	
	No.	%
Candida albicans	15,793	49.3
Candida glabrata	7,241	22.6
Candida parapsilosis	3,271	10.2
Candida tropicalis	1,489	4.6
Saccharomyces cerevisiae	612	2.0
Pichia kudriavzevii (*Candida krusei*)	554	1.7
Clavispora (*Candida*) *lusitaniae*	553	1.7
Cryptococcus neoformans	507	1.6
Candia dubliniensis	456	1.4
Meyerozyma (*Candida*) *guilliermondii*	357	1.1
Rhodotorula mucilaginosa	190	0.6
Kluyveromyces marxianus (*Candida kefyr*)	125	0.4
Pichia cactophila (*Candida inconspicua*)	101	0.3
Magnusiomyces capitatus	59	0.2
Candida nivariensis	56	0.2
Candida auris	45	0.1
Wickerhamomyces anomalus (*Candida pelliculosa*)	34	0.1
Trichosporon asahii	33	0.1
Yarrowia (*Candida*) *lipolytica*	32	0.1
Candida orthopsilosis	31	0.1
Candida metapsilosis	30	0.1
Candida africana	29	0.1
Wickerhamiella (*Candida*) *pararugosa*	24	0.1
Pichia (*Candida*) *norvegensis*	20	0.1
Cyberlindnera jadinii (*Candida utilis*)	20	0.1
Cyberlindnera (*Candida*) *fabianii*	19	0.1
Debaryomyces hansenii (*Candida famata*)	17	0.1
Meyerozyma caribbica (*Candida fermentati*)	17	0.1
Naganishia (*Cryptococcus*) *diffluens*	17	0.1
Lodderomyces elongisporus	15	<0.1
Dipodascus geotrichum	14	<0.1
Diutina (*Candida*) *rugosa*	14	<0.1
Pichia fermentans (*Candida lambica*)	14	<0.1
Naganishia albida (*Cryptococcus albidus*)	12	<0.1
Candida (*Nakaseomyces*) *bracarensis*	10	<0.1
Cutaneotrichosporon curvatum (*Cryptococcus curvatus*)	9	<0.1
Trichosporon mycotoxinivorans	8	<0.1
Candida blankii	7	<0.1
Candida zeylanoides	7	<0.1
Pichia kluyveri	7	<0.1
Rhodotorula dairenensis	7	<0.1
Diutina (*Candida*) *catenulata*	6	<0.1
Kazachstania telluris	6	<0.1
Metschnikowia pulcherrima	6	<0.1
Pichia mandshurica	6	<0.1
Rhodotorula glutinis	6	<0.1
Candida (*Pichia*) *eremophila*	5	<0.1
Cryptococcus uniguttulatus	5	<0.1
Candida intermedia	4	<0.1
Candida palmioleophila	3	<0.1
Kluyveromyces lactis	3	<0.1
Pichia galeiformis	3	<0.1
Rhodotorula slooffiae	3	<0.1
Scheffersomyces (*Candida*) *shehatae*	3	<0.1
Sporopachydermia cereana	3	<0.1
Starmerella (*Candida*) *magnoliae*	3	<0.1
Trichomonascus (*Candida/Stephanoascus*) *ciferrii*	3	<0.1
Wickerhamomyces onychis	3	<0.1
Apiotrichum (*Trichosporon*) *loubieri*	2	<0.1
Candida sake	2	<0.1

(Continued on next page)

TABLE 1 (Continued)

Organism (anamorph/previous name)	Prevalence of isolates received from 2002–2016	
	No.	%
Hanseniaspora guilliermondii	2	<0.1
Hanseniaspora uvarum	2	<0.1
Kodamaea (*Pichia*) *ohmeri*	2	<0.1
Metschnikowia reukaufii	2	<0.1
Pichia barkeri	2	<0.1
Pichia membranifaciens	2	<0.1
Others (single isolates of additional 26 species)[b]	26	0.1
Total	31,964	

[a]Where known, teleomorph names are provided (with anamorph or old name in parentheses) for those *Candida* species with known teleomorphs. Numbers of isolates and prevalence (%) are given.
[b]Others are single isolates of *Apiotrichum* (*Trichosporon*) *domesticum*, *Candida allociferrii*, *Candida boidinii*, *Candida diddensiae*, *Candida digboiensis*, *Candida ethanolicola*, *Candida melibiosica*, *C. norvegica*, *Candida pseudoglaebosa*, *Candida sojae*, *Candida sorboxylosa*, *Candida subashii*, *Debaryomyces nepalensis*, *Hanseniaspora opuntiae*, *Issatchenkia terricola*, *Kazachstania bovina*, *Kazachstania exigua*, *Kazachstania servazzii*, *Naganishia* (*Cryptococcus*) *liquefaciens*, *Rhodosporidiobolus nylandii*, *Saccharomycopsis fibuligera*, *Scheffersomyces* (*Candida*) *ergatensis*, *Sporidiobolus roseus*, *Starmerella* (*Candida*) *sorbosivorans*, *Starmera* (*Candida*) *stellimalicola*, and *Trichosporon dohaense*.

those having fluconazole MICs of ≥8 mg/liter. As this is a single-center study, we also included data for *C. albicans* isolates generated from 2002 to 2016 to allow comparisons with other European and U.S. reports (Table 2). In addition, although it was the 6th most common organism referred to the MRL (Table 1), *S. cerevisiae* was included in this analysis due to the elevated FLC MIC distributions observed with isolates of this organism (Table 2).

The fluconazole MIC data generated with *C. albicans* isolates from 2002 to 2016 (Table 2) were in excellent agreement with those of previously published studies that led to the development of both ECVs and CBPs with this organism-antifungal drug combination (19, 20). Using *C. albicans* interpretive breakpoints, the 82 less common yeast species could be crudely divided into two groups, as follows: those for which >20% of the isolates of a given species exhibited elevated FLC MICs (Table 2), and those for which <20% of the isolates had FLC MICs of ≥8 mg/liter (Table 3). A total of 45 species, encompassing 22 different genera, had fluconazole MIC distributions (or MIC values in the case of single isolates) suggestive of fluconazole resistance (Table 2). Of the 1,563 isolates that comprised these 45 species, 858 (54.9%) isolates had FLC MICs of ≥8 mg/liter (Table 2). Among these potentially fluconazole-resistant species, 13 species were represented by only a single isolate, with individual MIC values of 8 mg/liter (2 species), 16 mg/liter (5 species), 32 mg/liter (2 species), 64 mg/liter (1 species), or ≥64 mg/liter (3 species). An additional 20 species were represented by between 2 and 9 isolates, with potential fluconazole resistance rates of >50% in 14/20 of these species. For the remaining 12 species, where data from more than 10 isolates were available for analysis, resistance rates ranged from 24.9% (89/357 isolates) in *Meyerozyma* (*Candida*) *guilliermondii* to almost 100% (96% [97/101 isolates] with *Pichia cactophila* [*Candida inconspicua*] and 98.9% [188/190 isolates] with *Rhodotorula mucilaginosa*).

Based upon the same interpretive criteria for detecting potential fluconazole resistance, an additional 37 species (407 isolates) exhibited little or no resistance to this triazole antifungal (Table 3). While 14 of these 37 rare species were represented by single isolate, an additional 12 species were represented by 2 to 9 isolates, with data from in excess of 10 isolates available for analysis for the remaining 11 species. For 29/37 species, no resistant (FLC MIC, ≥8 mg/liter) isolates were encountered. For the remaining 8 species, resistance rates ranged from 0.8% (1/125 isolates) for *Kluyveromyces marxianus* (*Candida kefyr*) to 17.6% (3/17 isolates) for *Debaryomyces hansenii* (*Candida famata*) (Table 3).

TABLE 2 Fluconazole MIC distributions for 45 species of less common yeast where fluconazole resistance rates exceed 20% of isolates[a]

Species (no. of isolates)	Data for fluconazole MIC (mg/liter) of:											No. of resistant isolates/total no. of isolates (%)
	≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥64	
Candida albicans (15,302)	9,716	4,402	395	187	98	129	**139**	**90**	**43**	**38**	**65**	375/15,302 (2.5)
Candida allociferrii (1)								**1**				**1/1 (100)**
Candida blankii (7)		1				1	**2**	**1**	**1**	**1**		**5/7 (71)**
Candida boidinii (1)								**1**				**1/1 (100)**
Candida digboiensis (1)											**1**	**1/1 (100)**
Candida ethanolica (1)								1				**1/1 (100)**
Candida intermedia (4)					1	1		**1**	**1**			2/4 (50)
Candida palmioleophila (3)							**2**			**1**		**3/3 (100)**
Candida pseudoglaebosa (1)							**1**					**1/1 (100)**
Candida sorboxylosa (1)							**1**					**1/1 (100)**
Candida zeylanoides (7)				1	1	1	**1**	**1**		**1**	**1**	**4/7 (57)**
Cryptococcus uniguttulatus (5)									**2**	**2**	**1**	**5/5 (100)**
Cutaneotrichosporon curvatum (9)							**2**	**5**	**1**			**9/9(100)**
Debaryomyces nepalensis (1)									**1**			**1/1 (100)**
Dipodascus geotrichum (14)		1		1		2	**3**	**4**	**1**	**2**		10/14 (71.4)
Diutina catenulata (6)					3	1	**1**			**1**		2/6 (33)
Issatchenkia terricola (1)								**1**				**1/1 (100)**
Kodamaea ohmeri (2)						1			**1**			1/2 (50)
Magnusiomyces capitatus (59)				2	2	12	**24**	**16**	**2**		**1**	**43/59 (72.3)**
Meyerozyma guilliermondii (357)	2	4	14	25	104	119	**43**	**16**	**16**	**8**	**6**	89/357 (24.9)[b]
Naganishia albida (12)						4	**1**		**2**	**1**	**4**	**8/12 (66.7)**
Naganishia diffluens (17)			2		1	3	**5**	**5**			**1**	**11/17 (64.7)**
Naganishia liquefaciens (1)										**1**		**1/1 (100)**
Pichia barkeri (2)								**2**				**2/2 (100)**
Pichia cactophila (101)	1	2				1	**7**	**37**	**35**	**11**	**7**	**97/101 (96)**
Pichia eremophila (5)								**1**		**3**	**1**	**5/5 (100)**
Pichia fermentans (14)							**3**		**7**	**1**	**3**	**14/14 (100)**
Pichia galeiformis (3)										**2**	**1**	**3/3 (100)**
Pichia kluyveri (7)								**3**		**3**	**1**	**7/7 (100)**
Pichia mandshurica (6)	1		1	1			**1**		**1**		**1**	3/6 (50)
Pichia membranifaciens (2)							**1**				**1**	**2/2 (100)**
Pichia norvegensis (20)							**4**	**8**	**6**	**2**		**20/20 (100)**
Rhodosporidiobolus nylandii (1)											**1**	**1/1 (100)**
Rhodotorula dairenensis (7)									**1**	**5**	**1**	**7/7 (100)**
Rhodotorula glutinis (6)										**1**	**5**	**6/6 (100)**
Rhodotorula mucilaginosa (190)		1				1	**1**	**6**	**8**	**12**	**161**	**188/190 (98.9)**
Rhodotorula slooffiae (3)											**3**	**3/3 (100)**
Saccharomyces cerevisiae (612)	1	3	18	59	96	171	**147**	**78**	**28**	**10**	**1**	264/612 (43.1)
Scheffersomyces ergatensis (1)								**1**				**1/1 (100)**
Sporidiobolus roseus (1)											**1**	**1/1 (100)**
Starmerella magnoliae (3)			1						**1**		**1**	**2/3 (67)**
Starmerella sorbosivorans (1)									**1**			**1/1 (100)**
Trichosporon mycotoxinivorans (8)				1	2	2		**2**	**1**			3/8 (37.5)
Wickerhamiella pararugosa (24)				1	1	8	**8**	**3**	**1**	**2**		**14/24 (58)**
Wickerhamomyces onychis (3)				1	1		**1**					1/3 (33)
Yarrowia lipolytica (32)			1	1	7	11	**3**	**4**	**1**	**3**	**1**	12/32 (38)

[a]Resistance (R) was defined as an MIC of ≥8 mg/liter. The proportion of isolates of each species that fall above the *C. albicans* CBP (R) and the percentage resistance (%) are given, and the bold indicates that the %R was >50%. Data compiled from fluconazole susceptibility testing of isolates of *C. albicans* for the years 2002 to 2016 are also provided for comparison. All MICs were determined after 48 h of incubation. Isolate numbers above the *C. albicans* CBP are in bold.

[b]For *Meyerozyma* (*Candida*) *guilliermondii*, resistance rates fall to 12.9% (46/357 isolates) if the *C. guilliermondii* fluconazole ECV suggested by the CLSI (8 mg/liter) is applied to the MIC distribution as cutoff rather than the *C. albicans* CBP.

On the basis of relatively small numbers of isolates, we previously proposed that *C. nivariensis* (a member of the *Nakaseomyces* clade alongside *C. glabrata* and *C. bracarensis*) (22, 23) was an emerging pathogenic yeast which exhibited resistance to several of the triazole antifungal agents, including fluconazole (22), the resistance of which was disputed in several subsequent reports from other laboratories (reviewed in references 7 and 23). Here, analysis of the resistance profiles of the three species in the *Nakaseomyces* clade based on 15 years of MIC data confirms our previous observations for UK isolates (Table 4). Resistance rates for *C. nivariensis* with fluconazole or itraconazole were higher than for *C. glabrata* ($P < 0.01$, chi-square test) and similar for both species with voriconazole and posaconazole, and our laboratory employs the higher CLSI

TABLE 3 Fluconazole resistance rates for 37 species of uncommon yeast where resistance rates are less than 20%[a]

Organism	No. of resistant isolates/ total no. of isolates (%)
Apiotrichum domesticum	0/1 (0)
Apiotrichum loubieri	0/2 (0)
Candida africana	0/29 (0)
Candida bracarensis	0/10 (0)
Candida diddensiae	0/1 (0)
Candida melibiosica	0/1 (0)
Candida metapsilosis	1/30 (3.3)
Candida norvegica	0/1 (0)
Candida orthopsilosis	4/31 (12.9)
Candida sake	0/2 (0)
Candida sojae	0/1 (0)
Candida subhashii	0/1 (0)
Cyberlindnera fabianii	0/19 (0)
Cyberlindnera jadinii	2/20 (10)
Debaryomyces hansenii	3/17 (17.6)
Diutina rugosa	2/14 (14.3)
Hanseniaspora guilliermondii	0/2 (0)
Hanseniaspora opuntiae	0/1 (0)
Hanseniaspora uvarum	0/2 (0)
Kazachstania bovina	0/1 (0)
Kazachstania exigua	0/1 (0)
Kazachstania servazzii	0/1 (0)
Kazachstania telluris	1/6 (16.7)
Kluyveromyces lactis	0/3 (0)
Kluyveromyces marxianus	1/125 (0.8)
Lodderomyces elongisporus	0/15 (0)
Metschnikowia pulcherrima	0/6 (0)
Metschnikowia reukaufii	0/2 (0)
Meyerozyma caribbica	1/17 (5.9)
Saccharomycopsis fibuligera	0/1 (0)
Scheffersomyces shehatae	0/3 (0)
Sporopachydermia cereana	0/3 (0)
Starmera stellimalicola	0/1 (0)
Trichomonascus ciferrii	0/3 (0)
Trichosporon dohaense	0/1 (0)
Trichosporon asahii	1/33 (3.3)
Wickerhamomyces anomalus	3/34 (8.8)

[a]Resistance (R) was defined as an MIC of ≥8 mg/liter.

breakpoints established for *C. glabrata* with triazoles for the interpretation of *C. nivariensis* MICs to reflect this. Interestingly, based on the analysis of smaller numbers of isolates, frank triazole resistance does not seem to extend to the third member of the clade, *C. bracarensis*. Further work will be required to determine whether UK isolates of *C. nivariensis* do truly differ from their continental counterparts in terms of fluconazole susceptibility.

DISCUSSION

Here, we have presented fluconazole antifungal susceptibility data generated at the MRL over a 15-year period for 82 species of less common yeast isolated from clinical samples in the United Kingdom. For such organisms, the development of CBPs and ECVs is thwarted by insufficient isolate numbers for robust MIC distribution evaluation and a lack of sufficient clinical trial data for these rare emerging pathogens. The current study is not geared toward developing ECVs, since CLSI criteria stipulate that they require MIC data generated by at least three independent centers and the analysis of at least 100 independent isolates of a given species. However, it is hoped that the current study can contribute to the existing literature and aid in the future development of ECVs/CBPs. The MIC distributions reported here for fluconazole with *Candida albicans* and *C. glabrata* are very similar to those previously reported from large international studies involving the United States and continental Europe (19, 24, 25),

TABLE 4 Antifungal susceptibility profiles of members of the *Nakaseomyces* clade

Species by antifungal used (no. of isolates)	Data for antifungal MIC (mg/liter) of:														
	≤0.015	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64	%R[a]
Amphotericin B															
Candida glabrata (5,393)		2	21	249	1,162	3,215	739	**1**	**1**		**2**		**1**		0.07
Candida nivariensis (57)				2	11	40	4								0
Candida bracarensis (8)					3	1	4								0
Fluconazole															
Candida glabrata (6,879)			6		17	29	107	467	1,330	2,218	1,342	493	**420**	**450**	12.7
Candida nivariensis (56)					1		4	13	15	3		2	**6**	**11**	30.4
Candida bracarensis (10)							1	3	3	3					0
Itraconazole															
Candida glabrata (3,813)		53	73	205	485	1,091	1,167	261	215	**61**	**1**	**201**			6.9
Candida nivariensis (27)			3	7	3	4		2	4	**3**		**1**			14.8
Candida bracarensis (3)					1	2									
Voriconazole															
Candida glabrata (4,769)		255	445	891	1,264	**992**	**365**	**260**	**220**	**57**	**19**	**1**			40.3/19.5[b]
Candida nivariensis (37)		7	6	4	1	**4**	**2**	**2**	7	**3**	**1**				51.4/40.5[b]
Candida bracarensis (6)		1	2	1	2										0
Posaconazole															
Candida glabrata (382)		4	6	29	85	105	52	46	**20**	**4**	**8**	**23**			14.4
Candida nivariensis (26)			1	8	2	3	2	8	**2**						7.7
Candida bracarensis (1)				1											0
Anidulafungin															
Candida glabrata (1,110)	131	778	162	21	8	**4**	**1**	**1**	**2**	**1**					0.81
Candida nivariensis (7)	2	3		1	1										0
Candida bracarensis (7)	1	4	2												0

[a]Resistance rates (%R) are calculated based on CLSI CBPs or ECVs for *C. glabrata*.
[b]For voriconazole, two %R values were calculated according to the alternative ECVs of 0.25/0.5 mg/liter. Isolates above CBP/ECV are in bold, and modal MICs are underlined.

suggesting that UK isolates might be broadly comparable to their overseas counterparts. A limitation of the current study is that for a proportion of the yeast species considered here, isolate numbers were too low (<5 isolates per species) to reliably predict the likely fluconazole susceptibility profile of the species as a whole. However, in the absence of contradictory clinical data suggesting therapeutic benefits, additional MIC data from other centers for that particular organism, or susceptibility testing of an individual clinical isolate in question, these observations may be useful to clinicians confronted by extremely rare yeast species in deciding whether an antifungal agent other than fluconazole may be preferable.

For many of the other rare species analyzed here, isolate numbers were sufficient to generate more extensive fluconazole MIC distribution profiles. Such MIC distributions are clinically useful in identifying bimodal distributions indicative of subpopulations with acquired or intrinsic resistance. Similarly, the identification of antifungal agent-organism combinations where MIC ranges are always elevated compared to other species may aid in eliminating particular therapeutic approaches. A number of such combinations with several yeast species have become evident over the last decades and will not be discussed in detail here. When the CBP for fluconazole with *C. albicans* was applied to the MIC distributions presented here, a substantial number (45/82) of rarer yeast species exhibited MIC distributions that were clearly skewed toward resistance (Table 2). When only those species with >5 independent isolates were considered, fluconazole resistance rates based on the *C. albicans* CBP approaching 50 to 100% were seen with *Candida blankii*, *Dipodascus geotrichum* (*Geotrichum candidum*), *Magnusiomyces capitatus*, *Pichia cactophila*, *P. kluyveri*, *Pichia* (*Candida*) *norvegensis*, *Pichia fermentans* (*Candida lambica*), *Saccharomyces cerevisiae*, and various species of *Rhodotorula* (Table 2).

TABLE 5 Fluconazole resistance rates and numbers of isolates analyzed, MIC_{50} values, and MIC ranges from similar studies in China and France[a]

Organism	China %R (no. of isolates)[b]	MRL %R (no. of isolates)	MRL MIC_{50} (range) (mg/liter)	France MIC_{50} (range) (no. of isolates)[c]
Meyerozyma guilliermondii (*Candida guilliermondii*)	29.0 (186)	24.9 (357)	4 (0.125 to ≥64.0)	8 (1 to ≥64.0) (77)
Yarrowia lipolytica (*Candida lipolytica*)	69.4 (36)	38 (32)	4 (0.5 to ≥64)	4 (1 to 16) (18)
Pichia norvegensis (*Candida norvegensis*)	53.8 (13)	80 (20)	16 (0.25 to 64)	32 (8 to 64) (13)
Pichia cactophila (*C. inconspicua*)	87.5 (8)	96 (101)	32 (0.125 to ≥64)	16 (8 to 64) (26)
Wickerhamiella pararugosa (*Candida pararugosa*)	NA	58 (24)	16 (2 to 64)	8 (4 to 8) (5)
R. mucilaginosa	NA	97.9 (192)	>64 (0.125 to ≥64)	≥64 (32 to 64) (27)
S. cerevisiae	NA	42.3 (626)	4 (0.125 to ≥64)	8 (0.25 to 32) (35)

[a]Both studies defined fluconazole reduced susceptibility as isolates with MICs of >4 mg/liter.
[b]Comparative data compiled from reference 7.
[c]Comparative data compiled from reference 8.

Although the current study by necessity included only organisms isolated in the United Kingdom, we believe that the unusual fluconazole MIC distributions described here are likely to be relevant internationally. A recently published 5-year surveillance study of invasive candidiasis from China (7) also reported elevated fluconazole resistance rates for several of the same species that were highlighted in the current study (Table 5). Similarly, MIC_{50} values and MIC ranges generated from data collected during an exhaustive 12-year surveillance program in France (8) were very similar to those reported for the same species in the current report (Table 5). Moreover, a number of anecdotal case reports support our evidence for elevated MICs or fluconazole resistance in many of the species listed in Table 2, including *Yarrowia* (*Candida*) *lipolytica* (7, 8), *Pichia cactophila* and *Pichia norvegensis* (7, 8, 26–29), *C. blankii* (30), *Candida palmioleophila* (7, 31), *Candida zeylanoides* (29, 32, 33), *Diutina* (*Candida*) *catenulata* (7, 34), *Pichia fermentans* (29), *Pichia mandshurica* (8), *Debaryomyces hansenii* (*Candida famata*) (29, 35, 36), *Kodamaea ohmeri* (8, 35, 37–39), *Trichosporon* spp. (39–41), *Rhodotorula* spp. (8, 35, 41–44), *Debaryomyces nepalensis* (36), *Trichomonascus ciferrii* (39), *Saccharomyces cerevisiae* (8, 41, 45, 46), *Blastoschizomyces capitatus* (47–49), *Wickerhamiella* (*Candida*) *pararugosa* (8, 50, 51), *Dipodascus geotrichum* (41), *Starmerella* (*Candida*) *magnoliae* (7, 52), and *Meyerozyma guilliermondii* (7, 8, 53).

A skewed MIC distribution for a particular organism does not necessarily indicate resistance to that antifungal *per se*. It is well established that modal fluconazole MICs with *Candida glabrata* are significantly higher than those seen with many other *Candida* species, and yet infections with such isolates will still respond to treatment with higher doses of fluconazole. Indeed, such differences are reflected in the higher species-specific CBPs developed for *C. glabrata* with fluconazole. However, clinical support is accumulating to suggest that fluconazole at standard dosing might not be clinically indicated for a number of the organisms listed in Table 2, and that these skewed MICs may be reflective of true resistance rather than indicative of the need for alternate CBPs for such species. To date, fluconazole treatment failures and breakthrough infections during fluconazole prophylaxis have been described for *P. cactophila* and *P. norvegensis* (28, 54, 55), *Diutina catenulata* (33), *Trichosporon* spp. (39, 40), *Trichomonascus ciferrii* (39), *K. ohmeri* (39), *Rhodotorula* spp. (43, 44, 56), *Saccharomyces cerevisiae* (46), *Blastoschizomyces capitatus* (49), and *Meyerozyma guilliermondii* (57, 58). A word of caution, however, is necessary in this respect, since it is likely that case reports and small case series will overemphasize cases of poor outcome (8).

In summary, the current study has highlighted the occurrence of a large number of uncommon species of pathogenic yeast from very diverse taxonomic groups in clinical samples from the United Kingdom. Antifungal susceptibility testing performed in our laboratory on such isolates over a 15-year period indicated that a significant proportion of those uncommon species have fluconazole MIC distributions that are elevated compared to those of more common *Candida* species. While the primary aim of this study was to describe those uncommon yeast species for which fluconazole may not be the most appropriate antifungal agent, it also serves to emphasize the increasing

importance of robust yeast identification strategies in clinical laboratories that are exposed to an ever-expanding array of unusual pathogens.

Possible limitations of the current study. As discussed previously (59), this type of study is not intended to serve as an epidemiological survey of yeast species prevalence due to the reference nature of activity at the MRL, where isolate numbers are likely to be biased toward the more unusual organisms that have failed to be identified by the referring laboratories. Moreover, the current study excluded any isolates where fluconazole MIC determination was not requested by the referring laboratory. Methods for species identification have evolved considerably over the time period of the study, leading to the description of novel species and recognition of a number of species that were erroneously identified using older techniques. Here, we have tried to minimize the possible impact of potential erroneous identifications (IDs) on MIC distributions by excluding data for such species if the original isolates were not available for confirmation of identification by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). In the current study, we have not attempted to separate isolates based on the site of isolation (sterile versus superficial site), primarily because the isolation site was not available for many isolates, but also because the aim of the study was to evaluate the fluconazole susceptibility of rarer yeast species where isolate numbers from disseminated or deep infections are typically very low. However, even when combining isolates from superficial and deep sites, the total isolate numbers in many cases were less than the 15 per center recommended by EUCAST as being the minimum number required to define wild-type distributions. The inclusion of data for isolates from superficial sites (where prior antifungal exposure is more likely) and lack of information regarding the extent of prior exposure to antifungal therapy that might have resulted in acquired antifungal resistance for isolates from deep, usually sterile sites might be expected to skew MIC distributions toward nonsusceptibility. All of these factors might explain why MIC ranges for several species are unusually wide (see, for example, *S. cerevisiae* and *M. guilliermondii*) or apparently bimodal (*P. mandshurica, D. geotrichum*, and *C. nivariensis*). Finally, although all of the data presented here were obtained using CLSI broth microdilution methodologies, previous studies have demonstrated that MICs obtained by the CLSI and EUCAST methods show a reasonable correlation for *Candida* species and fluconazole (25). Moreover, even when different absolute MIC values are generated for a given species by CLSI and EUCAST methodologies, we would predict that both methods would similarly identify species with non-wild-type distributions, making the trends reported in the current study broadly applicable to laboratories that employ EUCAST methodologies.

MATERIALS AND METHODS

Clinical isolates for antifungal susceptibility testing. Between 2002 and 2016, 31,964 isolates of pathogenic yeast were submitted to the UK National Mycology Reference Laboratory (MRL), Bristol, UK, for determination of fluconazole MICs. Isolates included examples of common and rarer *Candida* species and a large number of other pathogenic yeasts from a wide variety of teleomorph genera (Table 1). Isolates were identified according to standard protocols employed at our laboratory as follows. Isolates received between 2002 and December 2007 were identified by a combination of Auxacolor 2/API 20C in conjunction with 26S rRNA gene sequencing (59); from January 2008 through May 2012, all isolates were identified by pyrosequencing of a portion of the internal transcribed spacer region 2 (5); and from May 2012 through December 2016, all isolates were identified by MALDI-TOF MS (6), with 26S rRNA gene sequencing in cases of identification failure. Although the exact antifungal drug susceptibility profile generated for each isolate depended on the site of isolation, virtually all isolates were tested against fluconazole. MICs were determined according to CLSI guidelines (60) by broth microdilution, as described below.

Antifungal susceptibility testing and determination of MICs. Antifungal drugs were obtained from their respective manufacturers as standard powders. Amphotericin B (Sigma Chemical Co., St. Louis, MO, USA) and anidulafungin and voriconazole (both Pfizer Central Research, Sandwich, UK) were dissolved in dimethyl sulfoxide. Itraconazole (Janssen Research Foundation, Beerse, Belgium) and posaconazole (Merck, Sharp and Dohme, Hoddesdon, UK) were dissolved in polyethylene glycol 400 (PEG 400) by heating to 70°C. Based on our laboratory experience, the solubility of these agents is better in PEG than in dimethyl sulfoxide (DMSO) (the solvent recommended by the CLSI), and precipitation of the antifungal agent upon freezing is reduced. Fluconazole (Pfizer Central Research) was suspended in sterile water. Serial 2-fold dilutions of the various drugs were prepared in RPMI 1640 medium (with L-glutamine, without bicarbonate; Sigma Chemical Co.) and buffered to pH 7.0 using a 0.165 M solution of morpho-

linepropanesulfonic acid (MOPS; Sigma Chemical Co.). The antifungal agents were tested over a range of final concentrations (0.015 to 8 μg/ml for anidulafungin, 0.03 to 16 μg/ml for amphotericin B, voriconazole, posaconazole, and itraconazole, and 0.125 to 64 μg/ml for fluconazole).

MICs were determined according to CLSI methodologies (60) in round-bottom 96-well plates with yeast blastospore suspensions prepared in saline and then diluted into RPMI 1640 and adjusted to final concentrations of $\sim 2.5 \times 10^3$ CFU/ml. Inoculated plates were incubated for 24 to 48 h at 35°C. MICs were read at 24 and/or 48 h (depending on the testing period) as the concentration of drug that elicited significant (approximately 50%) inhibition of growth compared with a drug-free control. Only MICs determined after 48 h are included in the current study. All assays included the control strains *Candida parapsilosis* NCPF 8334 (ATCC 22019) and *C. krusei* NCPF 3953 (ATCC 6258). Since CBPs and ECVs have been previously established for the more common *Candida* species, in the current analysis, MIC ranges were determined only for those *Candida* species that were less prevalent than *C. dubliniensis*. However, to allow comparison with other international studies, MIC data generated with *C. albicans* over the period of 2002 to 2016 ($n = 15{,}302$) were also included.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00211-19

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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